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# Effect of sub-optimal doses of fluoxetine plus estradiol on antidepressant-like behavior and hippocampal neurogenesis in ovariectomized rats

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**Summary** Estrogens and antidepressants synergize to reduce depressive symptoms and stimulate neurogenesis and neuroplastic events. The aim of this study was to explore whether the antidepressant-like effect induced by the combination of low doses of estradiol ($E_2$) and fluoxetine (FLX) involves changes in cell proliferation, early survival, morphology and dendrite complexity of hippocampal new-immature neurons.

The antidepressant-like effects of $E_2$ and/or FLX were evaluated by the forced swimming test (FST), cell proliferation was determined with the endogenous marker Ki67, survival of newborn cells was established with bromo-deoxiuridine (BrdU) and immature neurons were ascertained by doublecortin (DCX) labeling while their dendrite complexity was evaluated with Sholl analysis. Ovariectomized Wistar rats were randomly assigned to one of the following groups: Vehicle (saline/14 days + Oil/−8 h before FST); $E_2$ (saline/14 days + $E_2$ 2.5 or 10 μg/rat; −8 h before FST); FLX (1.25 or 10 mg/kg for 14 days + oil −8 h before FST), and FLX plus $E_2$ (FLX 1.25 mg/kg for 14 days + $E_2$ 2.5 μg/rat −8 h before FST).

The combination of sub-threshold doses of FLX plus $E_2$ produced antidepressant-like actions similar to those induced by FLX or $E_2$ given independently at optimal doses. Only FLX at an optimal dose and the combination of FLX plus $E_2$ increased cell proliferation, the number of

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DCX-labeled immature neurons and the complexity of their dendritic tree, suggesting that these events may be responsible for their antidepressant-like effect.


## 1. Introduction

Antidepressants are the main prescribed treatments for various mood disorders, including depression. At present, it is speculated that the therapeutic effect of antidepressants is due to structural and functional changes of several brain areas within the limbic system, such as the hippocampus. In line, increased hippocampal neurogenesis has been considered an integral effect of compounds with antidepressant-like effects (Santarelli et al., 2003; Malberg, 2004). Thus, several studies have shown that chronic treatment with antidepressants reverses the effect of stress on survival of newborn neurons by stimulating and restoring their dendrite-arborization (Norrholm and Ouimet, 2001; Malberg and Duman, 2003; Chen et al., 2006; Wang et al., 2008). In this sense, Wang et al. (2008) reported a high number of doublecortin-labeled (DCX) neurons with more complex dendritic trees after 28 days of fluoxetine (FLX) treatment, suggesting a hastening of dendritic maturation (Plumpe et al., 2006; Wang et al., 2008; Guirado et al., 2012). Furthermore, it has been proposed that FLX promotes the functional integration of newborn neurons by increasing the complexity of their dendritic trees (Wang et al., 2008; Guirado et al., 2012).

A way to explore new pharmacological strategies that shorten the onset of action of antidepressants is to combine them with estrogens (Estrada-Camarena et al., 2004; Estrada-Camarena et al., 2008). In this regard, it has been reported that estrogens, like estradiol ($E_2$) and ethinyl estradiol, drastically shorten the latency to the antidepressant-like effects of FLX and desipramine in the forced swimming test (FST) (Estrada-Camarena et al., 2004, 2008). Moreover, clinical data have shown that low doses of $E_2$ and antidepressants effectively reduce vasomotor (Joffe et al., 2014) and depressive symptoms without producing adverse effects (Nagata et al., 2005).

Several studies have reported that $E_2$, similarly to antidepressants (Malberg and Duman, 2003; Wang et al., 2008), increases neurogenesis in the dentate gyrus (DG) of young female rats (Tanapat et al., 1999; Banasr et al., 2001), inhibits cellular death (Tanapat et al., 1999; Barker and Galea, 2008) and enhances both the number of synapses and dendritic spines of hippocampal neurons (MacLusky et al., 2005; Velázquez-Zamora et al., 2012; Phan et al., 2012). Also, several reports have shown that the estrogen deficiency, induced by ovariectomy (OVX), regulates adult hippocampal neurogenesis (Tanapat et al., 1999, 2005; Banasr et al., 2001; Brock et al., 2010), decreases synaptic connectivity and reduces dendritic arborization (Day and Good, 2005; Wallace et al., 2006; Kataria et al., 2010). In fact, it has been shown that OVX combined with acute or chronic stress produces depressive-like behaviors associated with decreased newborn cell survival (Vega-Rivera et al., 2013, 2014). In addition, other studies propose that a reduction in synaptic plasticity contributes to the development of a depressive phenotype in animal models (Bessa et al., 2009; Mateus-Pinheiro et al., 2013). Considering that both $E_2$ and FLX exert an antidepressant-like effect and stimulate neurogenesis, it is possible that the effects of stress on both depressive-like behavior and neurogenesis could be reverted by the combination of $E_2$ plus FLX.

Thus, the aim of the present work was to study whether the combination of sub-threshold doses of $E_2$ and FLX, in parallel to its antidepressant-like effects induces changes in hippocampal neurogenesis (cell proliferation, early survival and/or the morphology and dendrite complexity of hippocampal doublecortin (DCX)-labeled newborn neurons) in ovariectomized rats.

## 2. Materials and methods

### 2.1. Animals

Female Wistar rats (250—300 g) were housed in standard laboratory cages under a 12-h light/12-h dark cycle (starting at 2200 h) at a temperature of $23 \pm 1\,^{\circ}C$ and with free access to food and water. One week after arrival, all rats were bilaterally OVX under 2,2,2-tribromoethanol (200 mg/kg, IP) anesthesia as previously described (Estrada-Camarena et al., 2003). All experimental procedures were performed in accordance with the Mexican official norm for animal care and handling (NOM-062-ZOO-1999) and approved by the local Institutional Ethics Committee of the National Institute of Psychiatry ''Ramón de la Fuente Muñiz'' and CINVESTAV-IPN.

### 2.2. Drugs

Fluoxetine hydrochloride (FLX; 1.25 or10 mg/kg; Bioquimed Laboratory, México) was dissolved in saline solution (0.9%) and administered IP (2 ml/kg) once a day for 14 days. 17β-estradiol ($E_2$, 2.5 or 10 μg/rat; Sigma-Aldrich, Toluca Mexico) was dissolved in corn oil and injected acutely (SC,0.2 ml/rat) 8 h before FST. Doses and latencies were selected from previous reports (Estrada-Camarena et al., 2004, 2008).

### 2.3. Forced swim test

The animals were placed in Plexiglass cylinders (20 cm in diameter and 46 cm tall) filled with a 30-cm layer of water at $23 \pm 2\,^{\circ}C$. The FST consisted of two sessions separated by 14 days (Detke et al., 1997; Estrada-Camarena et al., 2008). This period was chosen following previous reports indicating that the first session increased immobility that remains at least for 14 days (Detke et al., 1997; Estrada-Camarena et al., 2008; Vega-Rivera et al., 2013). In the first session rats were placed inside the cylinder for 15 min (pre-test)